

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 July 2001 (26.07.2001)

PCT

(10) International Publication Number
WO 01/53312 A1

(51) International Patent Classification⁷: **C07H 21/04**,
C12N 15/11, 15/63, 15/70, 15/82, 15/85, C07K 14/00

(21) International Application Number: PCT/US00/34263

(22) International Filing Date:
26 December 2000 (26.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/488,725	21 January 2000 (21.01.2000)	US
09/552,317	25 April 2000 (25.04.2000)	US
09/598,042	9 July 2000 (09.07.2000)	US
09/620,312	19 July 2000 (19.07.2000)	US
09/653,450	3 August 2000 (03.08.2000)	US
09/662,191	14 September 2000 (14.09.2000)	US
09/693,036	19 October 2000 (19.10.2000)	US
09/727,344	29 November 2000 (29.11.2000)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	09/488,725 (CIP)
Filed on	21 January 2000 (21.01.2000)
US	09/552,317 (CIP)
Filed on	25 April 2000 (25.04.2000)
US	09/598,042 (CIP)
Filed on	9 July 2000 (09.07.2000)
US	09/620,312 (CIP)
Filed on	19 July 2000 (19.07.2000)
US	09/653,450 (CIP)
Filed on	3 August 2000 (03.08.2000)
US	09/662,191 (CIP)
Filed on	14 September 2000 (14.09.2000)
US	09/693,036 (CIP)
Filed on	19 October 2000 (19.10.2000)
US	09/727,344 (CIP)
Filed on	29 November 2000 (29.11.2000)

(71) Applicant (for all designated States except US): **HYSEQ, INC.** [US/US]; 670 Almanor Avenue, Sunnyvale, CA 94086 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **TANG, Y., Tom** [US/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). **LIU, Chenghua** [CN/US]; 1125 Ranchero Way #14,

San Jose, CA 95117 (US). **ASUNDI, Vinod** [US/US]; 709 Foster City Boulevard, Foster City, CA 94404 (US). **CHEN, Rui-hong** [US/US]; 1031 Flying Fish Street, Foster City, CA 94404 (US). **MA, Yunqing** [CN/US]; 280 W. California Avenue #206, Sunnyvale, CA 94086 (US). **QIAN, Xiaohong, B.** [CN/US]; 3662 Tumble Way, San Jose, CA 95132 (US). **REN, Feiyan** [US/US]; 7703 Oak Meadow Court, Cupertino, CA 95014 (US). **WANG, Dunrui** [CN/US]; 932 La Palma, Milpitas, CA 95035 (US). **WANG, Jian-Rui** [CN/US]; 744 Stendhal Lane, Cupertino, CA 95014 (US). **WANG, Zhiwei** [CN/US]; 836 Alturas Avenue, B36, Sunnyvale, CA 94085 (US). **WEHRMAN, Tom** [US/US]; 3210 CCSR Mol Pharm, 269 W. Campus Drive, Stanford, CA 94305 (US). **XU, Chongjun** [CN/US]; 4918 Manitoba Drive, San Jose, CA 95130 (US). **XUE, Aidong, J.** [CN/US]; 1621 S. Mary Avenue, Sunnyvale, CA 94087 (US). **YANG, Yonghong** [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). **ZHANG, Jie** [CN/US]; 4930 Poplar Terrace, Campbell, CA 95008 (US). **ZHAO, Qing, A.** [CN/US]; 1556 Kooser Road, San Jose, CA 95118 (US). **ZHOU, Ping** [CN/US]; 1461 Japaul Lane, San Jose, CA 95132 (US). **GOODRICH, Ryle** [US/US]; 4896 Sandy Lane, San Jose, CA 95124 (US). **DRMANAC, Radoje, T.** [YU/US]; 850 East Greenwich Place, Palo Alto, CA 94303 (US).

(74) Agent: **ELRIFI, Ivor, R.**; Mintz, Levin, Cohn, Ferris, Glovsky, and Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

[Continued on next page]

(54) Title: NOVEL NUCLEIC ACIDS AND POLYPEPTIDES

(57) Abstract: The present invention provides novel nucleic acids, novel polypeptide sequences encoded by these nucleic acids and uses thereof.

WO 01/53312 A1



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

The present invention relates to a collection or library of at least one novel nucleic acid sequence assembled from expressed sequence tags (ESTs) isolated mainly by sequencing by hybridization (SBH), and in some cases, sequences obtained from one or more public databases. The invention relates also to the proteins encoded by such polynucleotides, along with therapeutic, 5 diagnostic and research utilities for these polynucleotides and proteins. These nucleic acid sequences are designated as SEQ ID NO: 1-1786 and 3573-5358. The polypeptides sequences are designated SEQ ID NO: 2n (wherein n = 1 to 20). The nucleic acids and polypeptides are provided in the Sequence Listing. In the nucleic acids provided in the Sequence Listing, A is adenosine; C is cytosine; G is guanine; T is thymine; and N is any of the four bases. In the amino acids provided in 10 the Sequence Listing, * corresponds to the stop codon.

The nucleic acid sequences of the present invention also include, nucleic acid sequences that hybridize to the complement of SEQ ID NO:1-1786 and 3573-5358 under stringent hybridization conditions; nucleic acid sequences which are allelic variants or species homologues of any of the nucleic acid sequences recited above, or nucleic acid sequences that encode a peptide comprising a 15 specific domain or truncation of the peptides encoded by SEQ ID NO:1-1786 and 3573-5358. A polynucleotide comprising a nucleotide sequence having at least 90% identity to an identifying sequence of SEQ ID NO:1-1786 and 3573-5358 or a degenerate variant or fragment thereof. The identifying sequence can be 100 base pairs in length.

The nucleic acid sequences of the present invention also include the sequence information 20 from the nucleic acid sequences of SEQ ID NO:1-1786 and 3573-5358. The sequence information can be a segment of any one of SEQ ID NO:1-1786 and 3573-5358 that uniquely identifies or represents the sequence information of SEQ ID NO:1-1786 and 3573-5358.

A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or identifying information of each sequence can be provided on 25 a nucleic acid array. In one embodiment, segments of sequence information is provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array can be designed to detect full-match or mismatch to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

This invention also includes the reverse or direct complement of any of the nucleic acid 30 sequences recited above; cloning or expression vectors containing the nucleic acid sequences; and host cells or organisms transformed with these expression vectors. Nucleic acid sequences (or their reverse or direct complements) according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology, such as use as hybridization probes, use as primers for PCR, use in an array, use in computer-readable media, use in sequencing

full-length genes, use for chromosome and gene mapping, use in the recombinant production of protein, and use in the generation of anti-sense DNA or RNA, their chemical analogs and the like.

In a preferred embodiment, the nucleic acid sequences of SEQ ID NO:1-1786 and 3573-5358 or novel segments or parts of the nucleic acids of the invention are used as primers in expression assays that are well known in the art. In a particularly preferred embodiment, the nucleic acid sequences of SEQ ID NO:1-1786 and 3573-5358 or novel segments or parts of the nucleic acids provided herein are used in diagnostics for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., *Science* 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

10 The isolated polynucleotides of the invention include, but are not limited to, a polynucleotide comprising any one of the nucleotide sequences set forth in SEQ ID NO:1-1786 and 3573-5358; a polynucleotide comprising any of the full length protein coding sequences of SEQ ID NO:1-1786 and 3573-5358; and a polynucleotide comprising any of the nucleotide sequences of the mature protein coding sequences of SEQ ID NO:1-1786 and 3573-5358. The polynucleotides of the 15 present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent hybridization conditions to (a) the complement of any one of the nucleotide sequences set forth in SEQ ID NO:1-1786 and 3573-5358; (b) a nucleotide sequence encoding any one of the amino acid sequences set forth in the Sequence Listing; (c) a polynucleotide which is an allelic variant of any polynucleotides recited above; (d) a polynucleotide which encodes a species homolog 20 (e.g. orthologs) of any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of any of the polypeptides comprising an amino acid sequence set forth in the Sequence Listing.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising any of the amino acid sequences set forth in the Sequence Listing; or the corresponding 25 full length or mature protein. Polypeptides of the invention also include polypeptides with biological activity that are encoded by (a) any of the polynucleotides having a nucleotide sequence set forth in SEQ ID NO:1-1786 and 3573-5358; or (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or immunologically active variants of any of the polypeptide sequences in the Sequence Listing, and "substantial 30 equivalents" thereof (e.g., with at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% amino acid sequence identity) that preferably retain biological activity are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (e.g. host cells) of the invention.

The invention also provides compositions comprising a polypeptide of the invention. Polypeptide compositions of the invention may further comprise an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

5 The invention also provides host cells transformed or transfected with a polynucleotide of the invention.

10 The invention also relates to methods for producing a polypeptide of the invention comprising growing a culture of the host cells of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the polypeptide from the culture or from the host cells. Preferred embodiments include those in which the protein produced by such process is a mature form of the protein.

15 Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers, or primers, for PCR, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of anti-sense DNA or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, e.g., *in situ* hybridization.

20 In other exemplary embodiments, the polynucleotides are used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., *Science* 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

25 The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide. Such antibodies, particularly monoclonal antibodies, are useful for detecting or quantitating the polypeptide in tissue. The polypeptides of the invention can also be used as molecular weight markers, and as a food supplement.

30 Methods are also provided for preventing, treating, or ameliorating a medical condition which comprises the step of administering to a mammalian subject a therapeutically effective amount of a composition comprising a polypeptide of the present invention and a pharmaceutically acceptable carrier.

35 In particular, the polypeptides and polynucleotides of the invention can be utilized, for example, in methods for the prevention and/or treatment of disorders involving aberrant protein expression or biological activity.

The present invention further relates to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample. Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders as recited herein and for the identification of subjects exhibiting a predisposition to such conditions. The invention provides

5 a method for detecting the polynucleotides of the invention in a sample, comprising contacting the sample with a compound that binds to and forms a complex with the polynucleotide of interest for a period sufficient to form the complex and under conditions sufficient to form a complex and detecting the complex such that if a complex is detected, the polynucleotide of interest is detected. The invention also provides a method for detecting the polypeptides of the

10 invention in a sample comprising contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex and detecting the formation of the complex such that if a complex is formed, the polypeptide is detected.

The invention also provides kits comprising polynucleotide probes and/or monoclonal antibodies, and optionally quantitative standards, for carrying out methods of the invention. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds that modulate (i.e., increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited herein. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (e.g., bind to) the polypeptides of the invention. The invention provides a method for identifying a compound that binds to the polypeptides of the invention comprising contacting the compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and detecting the complex by detecting the reporter gene sequence expression such that if expression of the reporter gene is detected the compound binds to a polypeptide of the invention is identified.

The methods of the invention also provides methods for treatment which involve the administration of the polynucleotides or polypeptides of the invention to individuals exhibiting symptoms or tendencies. In addition, the invention encompasses methods for treating diseases or disorders as recited herein comprising administering compounds and other substances that modulate the overall activity of the target gene products. Compounds and other substances can

protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or 5 enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new 10 sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively 15 selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine 20 phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. 25 PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

4.6 POLYPEPTIDES OF THE INVENTION

The isolated polypeptides of the invention include, but are not limited to, a polypeptide 30 comprising: the amino acid sequences set forth as any one of SEQ ID NO:1787-3572 and 5359-7144 or an amino acid sequence encoded by any one of the nucleotide sequences SEQ ID NO:1-1786 and 3573-5358 or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides preferably with biological or immunological activity that are encoded by: (a) a polynucleotide having any one of the nucleotide sequences set forth in SEQ ID 35 NO:1-1786 and 3573-5358 or (b) polynucleotides encoding any one of the amino acid sequences

set forth as SEQ ID NO:1787-3572 and 5359-7144 or (c) polynucleotides that hybridize to the complement of the polynucleotides of either (a) or (b) under stringent hybridization conditions. The invention also provides biologically active or immunologically active variants of any of the amino acid sequences set forth as SEQ ID NO:1787-3572 and 5359-7144 or the corresponding 5 full length or mature protein; and "substantial equivalents" thereof (e.g., with at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, typically at least about 95%, more typically at least about 98%, or most typically at least about 99% amino acid identity) that retain biological activity. Polypeptides encoded by allelic variants may have a similar, increased, or decreased activity compared to polypeptides 10 comprising SEQ ID NO:1787-3572 and 5359-7144.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., *Bio/Technology* 10, 773-778 (1992) and in R. S. McDowell, et al., *J. Amer. 15 Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites.

The present invention also provides both full-length and mature forms (for example, without a signal sequence or precursor sequence) of the disclosed proteins. The protein coding 20 sequence is identified in the sequence listing by translation of the disclosed nucleotide sequences. The mature form of such protein may be obtained by expression of a full-length polynucleotide in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein is also determinable from the amino acid sequence of the full-length form. Where proteins of the present invention are membrane bound, soluble forms of the proteins are also 25 provided. In such forms, part or all of the regions causing the proteins to be membrane bound are deleted so that the proteins are fully secreted from the cell in which they are expressed.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

The present invention further provides isolated polypeptides encoded by the nucleic acid 30 fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.*, an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. This technique is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

The invention also relates to methods for producing a polypeptide comprising growing a culture of host cells of the invention in a suitable culture medium, and purifying the protein from the cells or the culture in which the cells are grown. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, or from a lysate prepared from the host cells and further purified. Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography. See, e.g., Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*; Ausubel et al., *Current Protocols in Molecular Biology*. Polypeptide fragments that

retain biological/immunological activity include fragments comprising greater than about 100 amino acids, or greater than about 200 amino acids, and fragments that encode specific protein domains.

The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for e.g., small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the peptides of the invention or molecules capable of binding to the peptides may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for SEQ ID NO:1787-3572 and 5359-7144.

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications, in the peptide or DNA sequence, can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Pat. No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein. Regions of the protein that are important for the protein function can be determined by various methods known in the art including the alanine-scanning method which involved systematic substitution of single or strings of amino acids with alanine, followed by testing the resulting alanine-containing variant for biological activity. This type of analysis determines the importance of the substituted amino acid(s) in biological activity. Regions of the protein that are important for protein function may be determined by the eMATRIX program.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and are useful for screening or other immunological

methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing 5 an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBacTM kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present 10 invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification 15 of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearlTM or Cibacrom blue 3GA SepharoseTM; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

20 Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX), or as a His tag. Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and Invitrogen, 25 respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("FLAG[®]") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP- HPLC) 30 steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The polypeptides of the invention include analogs (variants). This embraces fragments, as well as peptides in which one or more amino acids has been deleted, inserted, or substituted. Also, analogs of the polypeptides of the invention embrace fusions of the polypeptides or modifications of the polypeptides of the invention, wherein the polypeptide or analog is fused to another moiety or moieties, e.g., targeting moiety or another therapeutic agent. Such analogs may exhibit improved properties such as activity and/or stability. Examples of moieties which may be fused to the polypeptide or an analog include, for example, targeting moieties which provide for the delivery of polypeptide to pancreatic cells, e.g., antibodies to pancreatic cells, antibodies to immune cells such as T-cells, monocytes, dendritic cells, granulocytes, etc., as well as receptor and ligands expressed on pancreatic or immune cells. Other moieties which may be fused to the polypeptide include therapeutic agents which are used for treatment, for example, immunosuppressive drugs such as cyclosporin, SK506, azathioprine, CD3 antibodies and steroids. Also, polypeptides may be fused to immune modulators, and other cytokines such as alpha or beta interferon.

15

4.6.1 DETERMINING POLYPEPTIDE AND POLYNUCLEOTIDE IDENTITY AND SIMILARITY

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, FASTA (Altschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990), PSI-BLAST (Altschul S.F. et al., Nucleic Acids Res. vol. 25, pp. 3389-3402, herein incorporated by reference), eMatrix software (Wu et al., J. Comp. Biol., Vol. 6, pp. 219-235 (1999), herein incorporated by reference), eMotif software (Nevill-Manning et al, ISMB-97, Vol. 4, pp. 202-209, herein incorporated by reference), pFam software (Sonhammer et al., Nucleic Acids Res., Vol. 26(1), pp. 320-322 (1998), herein incorporated by reference) and the Kyte-Doolittle hydrophobicity prediction algorithm (J. Mol Biol, 157, pp. 105-31 (1982), incorporated herein by reference). The BLAST programs are publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990)).

4.7 CHIMERIC AND FUSION PROTEINS

The invention also provides chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises a polypeptide of the invention operatively linked to

SEQ ID NO: of full- length nucleotide sequence	SEQ ID NO: of full- length peptide sequence	SEQ ID NO: of contig nucleotide sequence	SEQ ID NO: of contig peptide sequence	Priority docket number_ corresponding SEQ ID NO: in priority application	SEQ ID NO:in U.S.S.N. 09/488,725
1734	3520	5306	7092	784CIP2D_82	9867
1735	3521	5307	7093	784CIP2D_83	10010
1736	3522	5308	7094	784CIP2D_84	10011
1737	3523	5309	7095	784CIP2D_85	10052
1738	3524	5310	7096	784CIP2D_86	10057
1739	3525	5311	7097	784CIP2D_87	10085
1740	3526	5312	7098	784CIP2D_89	10139
1741	3527	5313	7099	784CIP2D_90	10142
1742	3528	5314	7100	784CIP2D_92	10165
1743	3529	5315	7101	784CIP2D_93	10173
1744	3530	5316	7102	784CIP2D_94	10173
1745	3531	5317	7103	784CIP2D_95	10273
1746	3532	5318	7104	784CIP2E_1	3121
1747	3533	5319	7105	784CIP2E_2	3628
1748	3534	5320	7106	784CIP2E_4	3673
1749	3535	5321	7107	784CIP2E_5	4018
1750	3536	5322	7108	784CIP2E_6	4467
1751	3537	5323	7109	784CIP2E_7	4865
1752	3538	5324	7110	784CIP2E_8	4916
1753	3539	5325	7111	784CIP2E_9	4923
1754	3540	5326	7112	784CIP2E_10	4926
1755	3541	5327	7113	784CIP2E_11	4962
1756	3542	5328	7114	784CIP2E_12	4963
1757	3543	5329	7115	784CIP2E_13	4964
1758	3544	5330	7116	784CIP2E_14	4988
1759	3545	5331	7117	784CIP2E_15	5835
1760	3546	5332	7118	784CIP2E_16	7682
1761	3547	5333	7119	784CIP2E_17	7682
1762	3548	5334	7120	784CIP2E_18	7699
1763	3549	5335	7121	784CIP2E_19	7707
1764	3550	5336	7122	784CIP2E_20	7707
1765	3551	5337	7123	784CIP2E_21	7752
1766	3552	5338	7124	784CIP2E_22	8357
1767	3553	5339	7125	784CIP2E_23	9065
1768	3554	5340	7126	784CIP2E_24	9324
1769	3555	5341	7127	784CIP2F_1	2976
1770	3556	5342	7128	784CIP2F_2	3559
1771	3557	5343	7129	784CIP2F_3	4021
1772	3558	5344	7130	784CIP2F_4	4474
1773	3559	5345	7131	784CIP2F_5	4566
1774	3560	5346	7132	784CIP2F_6	4705
1775	3561	5347	7133	784CIP2F_7	4707
1776	3562	5348	7134	784CIP2F_8	4712
1777	3563	5349	7135	784CIP2F_9	5008
1778	3564	5350	7136	784CIP2F_10	5009
1779	3565	5351	7137	784CIP2F_11	5015
1780	3566	5352	7138	784CIP2F_12	5015
1781	3567	5353	7139	784CIP2F_13	7724
1782	3568	5354	7140	784CIP2F_14	7725
1783	3569	5355	7141	784CIP2F_15	8828
1784	3570	5356	7142	784CIP2F_16	8830
1785	3571	5357	7143	784CIP2F_17	9739
1786	3572	5358	7144	784CIP2F_18	9896

TRADOCs:1416247.1(%CS70II.DOC)

275	280	285
Ala Val Ile Gln Ser Lys Asp Ala Thr Ile Gln Glu Leu Lys Glu Lys		
290	295	300
Ile Ala Tyr Leu Glu Ala Glu Asn Leu Glu Met His Asp Arg Met Glu		
305	310	315
His Leu Ile Glu Lys Gln Ile Ser His Gly Asn Phe Ser Thr Gln Ala		
325	330	335
Arg Ala Lys Thr Glu Asn Pro Gly Ser Ile Arg Ile Ser Lys Pro Pro		
340	345	350
Ser Pro Lys Pro Met Pro Val Ile Arg Val Val Glu Thr *		
355	360	365

<210> 3567
 <211> 151
 <212> PRT
 <213> Homo sapiens

<400> 3567		
Met Ser Thr Val Pro Gly Gly Ser Arg His Ser Leu Gly Ile Gln Val		
1	5	10
Arg Gly Gly Trp Gly Val Thr Gly Gly Glu Glu Glu Ser Leu Thr Val		
20	25	30
Pro Val Ala Asp Thr Trp Gln Ala Gly Ser Phe Lys Val Ala Thr Gln		
35	40	45
Glu Arg Asn Pro Gln Arg Ala Gln Met Arg Leu Arg Arg Gln Lys Lys		
50	55	60
Gly Val Val Pro Phe Leu Gly Asp Phe Leu Thr Glu Leu Gln Arg Leu		
65	70	75
Asp Ser Ala Ile Pro Asp Asp Leu Asp Gly Asn Thr Asn Lys Arg Ser		
85	90	95
Lys Glu Val Arg Val Leu Gln Glu Met Gln Leu Leu Gln Val Ala Ala		
100	105	110
Met Asn Tyr Arg Leu Arg Pro Leu Glu Lys Phe Val Thr Tyr Phe Thr		
115	120	125
Arg Met Glu Gln Leu Ser Asp Lys Glu Ser Tyr Lys Leu Ser Cys Gln		
130	135	140
Leu Glu Pro Glu Asn Pro *		
145	150	

<210> 3568
 <211> 126
 <212> PRT
 <213> Homo sapiens

<400> 3568		
Met Ser Thr Val Pro Gly Gly Ser Arg His Ser Leu Gly Ile Gln Val		
1	5	10
Arg Gly Gly Trp Gly Val Thr Gly Gly Glu Glu Glu Ser Leu Thr Val		
20	25	30
Pro Val Ala Asp Thr Trp Gln Ala Gly Ser Phe Lys Val Ala Thr Gln		
35	40	45
Glu Arg Asn Pro Gln Arg Ala Gln Met Arg Leu Arg Arg Gln Lys Lys		
50	55	60
Gly Asn Thr Asn Lys Arg Ser Lys Glu Val Arg Val Leu Gln Glu Met		
65	70	75
Gln Leu Leu Gln Val Ala Ala Met Asn Tyr Arg Leu Arg Pro Leu Glu		
85	90	95
Lys Phe Val Thr Tyr Phe Thr Arg Met Glu Gln Leu Ser Asp Lys Glu		
100	105	110
Ser Tyr Lys Leu Ser Cys Gln Leu Glu Pro Glu Asn Pro *		